any mode of administration, any gene transfer vector carrying any nitric oxide synthase gene, wild type or mutant, under the control of any expression control element. The attached Declaration Under 37 C.F.R. § 1.132 by Dr. Stefan Janssens, one of the inventors, addresses this rejection, and is summarized here.

Several arguments were raised in support of the enablement rejection. The first was that the specification does not enable the use of all mutants of all NOS genes in the claimed methods. In answer, the Declaration explains that the specification provided sufficient information regarding appropriate amino acid mutations, as well as a method for testing NOS mutants for the activity necessary to practice the claimed methods. Moreover, according to the Declaration much information about the structure and function of the domains of the NOS isoforms were well known. Several NOS mutants had been prepared, and the Declaration cites papers that disclose a NOS mutant that retains the necessary biological activity, and several NOS mutants that do not. The Declaration asserts that, in view of this information, one skilled in the art would be able to make and use NOS mutants according to the claimed methods without undue experimentation. Therefore, Applicants respectfully request that this ground for the enablement rejection be withdrawn.

The Examiner's second argument in support of the enablement rejection is that the specification, combined with information known in the art as of the priority date, were insufficient to enable the use of any gene transfer vector with any expression control element by any mode of administration, because of the challenges in transducing particular tissue types, and obtaining and maintaining sufficient expression of the transgene. The Examiner contends that because certain viral vectors can efficiently transduce only dividing cells, they cannot be used to transfer a gene to lung tissue, which contains some non-dividing cell types.

According to the Declaration, the specification discloses gene transfer vectors, in addition to the adenoviral vector disclosed in the Examples, e.g., retroviral vectors and liposomes. The Declaration also states that other vectors for transferring genes to cells in lung tissue, such as recombinant adeno-associated virus, and an HIV-based lentiviral vector, were known and had been successfully used to deliver genes to cell types found in the lung. It cites articles reporting the use of these vectors to transfer genes to cells in pulmonary tissue, such as cells in the pulmonary vasculature.

In addition, according to the Declaration, several non-viral gene transfer vectors for efficient delivery to pulmonary tissue were also known, e.g., (1) liposomes; (2) targeted liposomes, which achieve tissue specific uptake by incorporating molecules, such as immunoglobulins, into the liposome; and (3) non-liposomal targeted gene transfer using DNA-conjugate complexes to exploit cell-internalization pathways, or by conjugating the transgene to a cell surface receptor specific for lung tissue. Papers reviewing these vectors, and methods for increasing the efficiency of liposomal vectors are cited. The Declaration concludes that in view of information disclosed in the specification and known in the art as of the priority date regarding vectors for carrying transgenes, the selection of vectors that could be used in the claimed methods of inducing pulmonary vasodilation and treating pulmonary hypertension would not require undue experimentation by one of skill in the art. Consequently, Applicants respectfully request that this ground for the enablement rejection be withdrawn.

With regard to expression control elements for controlling the expression of NOS in cells in lung tissue, the Declaration reviews those disclosed in the specification, e.g., the constitutive cytomegalovirus early gene promoter/enhancer and the SV40 polyadenylation signal sequence, as well as several other expression control elements, e.g., the long terminal repeat of the Rous

sarcoma virus, the adeno-associated virus p_5 promoter, the adeno-associated virus ITR sequence, the endothelin-1 promoter, the eNOS promoter, the surfactant D promoter, the human surfactant protein-C (SP-C) promoter sequences, and the SM 22 alpha promoter. Papers disclosing the use of these expression control elements to direct the expression of a transgene in cells found in lung tissue are also cited in the Declaration. In view of the information disclosed in the specification, and known in the art as of the priority date, the Declaration asserts that the selection of expression control elements to direct transgene expression in lung tissue, would not require undue experimentation by one of skill in the art. Applicants respectfully request that this ground for the enablement rejection be withdrawn.

Furthermore, the Declaration discloses that routes of gene delivery to lung tissue other than aerosol delivery are disclosed in the specification, e.g., intratracheal delivery, intravenous delivery, intraarterial delivery, and *ex vivo* delivery, and that these methods were known and used as of the priority date. The Declaration reviews papers in which these methods were used, and notes that catheters were used routinely to deliver drugs to selected areas of the vasculature, and had been used to deliver transgenes to human patients in clinical trials. Finally, the Declaration concludes that, in view of the information disclosed in the specification and known as of the priority date, it would not require undue experimentation for one of skill in the art to select and use an appropriate method to deliver a gene transfer vector to the lung. Applicants respectfully request that this ground for the enablement rejection be withdrawn.

The Examiner's next contention is that the claim relating to the administration of an immunosuppressive agent to a mammal also receiving a vector carrying a NOS gene to induce pulmonary vasodilation is not enabled because cyclosporin A, an immunosuppressive agent explicitly named in the specification, has been reported by Roullet *et al.*, *J. Clin. Invest. 93*:2244-

50 (1993), to increase systemic blood pressure when administered. In view of this report, the Examiner has argued that one of skill in the art would expect that cyclosporin A would counter the effect of the NOS gene, and thus, would not want to administer both to a patient.

The paper cited by the Examiner, Roullet *et al.*, reports that cyclosporin A (CysA) increased systemic blood pressure in Sprague Dawley rats, and that there were some differences in mesenteric artery reactivity between CysA treated vessels and control vessels. However, the Declaration states that pulmonary and systemic (e.g., mesenteric) blood vessels are known to significantly differ in vasoreactivity to various stimuli, therefore, data observed in mesenteric vessels should not be extrapolated to pulmonary vessels. Moreover, according to the Declaration, reports on the effect of cyclosporin on pulmonary vascular resistance in human patients conflict. In addition, the Declaration suggests that it may be possible to avoid possible systemic effects of CysA by delivering it directly to the lungs via aerosol. In view of this information, the Declaration asserts that one of skill in the art, reading Roullet *et al.*, would not assume that the action of cysA is diametrically opposed to NOS, and should not be coadministered with NOS for the induction of pulmonary vasodilation.

In addition, according to the Declaration, several immunosuppressive agents not usually associated with pulmonary hypertension, such as methotrexate, and prednisone, were known in the art at the time of the priority date, and only routine experimentation would be required to select one for use in the claimed method of inducing pulmonary vasodilation. For this reason and those stated above, the Examiner has failed to establish that the claim relating to the administration of an immunosuppressive agent to a mammal also receiving a vector carrying a NOS gene to induce pulmonary vasodilation is not enabled. Therefore, Applicants respectfully request that this ground for rejection be withdrawn.

The Examiner's final contention is that the claimed methods of treating pulmonary hypertension are enabled only for use in the rat, because the rat is allegedly not a good model for pulmonary hypertension in humans. In support, the Examiner relies on a statement in Heath, *Eur. Respir. Rev. 3:* 555-58 (1993) that suggests that the rat is not a good model for pulmonary hypertension in humans because, according to Heath, it does not undergo the migration of smooth muscle cells into the intima of blood vessels that occurs in the disease in humans.

The Declaration states that the rat pulmonary hypertension model is an appropriate model for pulmonary hypertension in humans, because rat pulmonary blood vessels do undergo certain structural alterations seen in this disease in humans, e.g., the extension of muscle cells into smaller and more peripheral arteries, and smooth muscle cell proliferation, differentiation and hypertrophy. In addition, according to the Declaration, data collected from Dr. Janssens' post-filing experiments show that the transfer of a NOS gene to the lung suppressed the muscular hypertrophy in a rat model of pulmonary hypertension. Moreover, the Declaration states that certain drugs first found to be pulmonary vasodilators in rats, including inhaled nitric oxide and beraprost, have also been found to be pulmonary vasodilators in humans with primary pulmonary hypertension. Thus, as is asserted in the Declaration, the shared vascular structural changes seen in rats and humans, Dr. Janssens' results showing an affect of NOS on muscular hypertrophy, and the previous correlation between results from experiments done in the rat and results in humans for certain drugs, all support the use of the rat as a model for pulmonary hypertension in humans. Therefore, Heath *et al.* fails to establish that the application enables the treatment of hypertension only in rats, and Applicants respectfully request that this ground for rejection be withdrawn.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. It is believed that a full and complete reply has been made to the outstanding Office Action and, as such, the claims are in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,

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